

Estrogen and neuroprotection: higher constitutive expression of glutaredoxin in female mice offers protection against MPTP-mediated neurodegeneration

RAJAPPA S. KENCHAPPA,^{*,†,1} LATHA DIWAKAR,^{*,†,1} JAYASREE ANNEPU,[†] AND VIJAYALAKSHMI RAVINDRANATH^{*,†,2}

^{*}Division of Molecular and Cellular Neuroscience, National Brain Research Centre, Nainwal Mode, Manesar, India; and [†]Department of Neurochemistry, National Institute of Mental Health and Neurosciences, Bangalore, India

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SPECIFIC AIMS

MPTP, a dopaminergic neurotoxin, acts through inhibition of mitochondrial complex I, which produces Parkinson's disease-like symptoms in primates, including humans. Epidemiological studies have shown that the risk/incidence of Parkinson's disease in men is nearly twice that of women. Although the neuroprotective effect of estrogen is known, its mode of action is not well understood. We therefore examined the molecular mechanisms underlying the neuroprotection seen in female mice using MPTP as a model dopaminergic neurotoxin.

PRINCIPAL FINDINGS

1. Female mice are resistant to MPTP neurotoxicity

Male and female mice were administered of MPTP (30 mg/kg body wt, s.c) as a single dose or daily for 8 days and complex I activity was measured in striatum and midbrain. After acute and chronic administration of MPTP, complex I activity was significantly decreased in striatum and midbrain of male mice while there was no change in females (Fig. 1a). After 8 days of chronic MPTP exposure, the brains were removed from male and female mice and coronal sections were cut from the anteroposterior middle area of the substantia nigra para compacta. Morphological evaluation of dopaminergic neurons was performed by immunostaining for tyrosine hydroxylase. Tyrosine hydroxylase staining was nearly undetectable in the substantia nigra of male mice, indicating loss of dopaminergic neurons; in female mice the immunostaining was similar to controls, indicating that the dopaminergic neurons in females were unaffected by chronic administration of MPTP (Fig. 1b).

2. Total GSH levels and glutaredoxin (Grx1) expression are unaltered in female mouse brain after MPTP administration

In male mice, total GSH levels in striatum and midbrain decreased significantly after a single dose of MPTP but were unaffected in female mice. However, GSSG levels in the striatum and midbrain of female mice significantly increased 4 h after MPTP treatment (101% and 65% higher than corresponding controls, respectively), indicating the generation of oxidative stress in female mice. We had earlier demonstrated that Grx1 is important for maintaining the functional integrity of complex I and its up-regulation is required for the recovery of mitochondrial complex I activity after a single dose of MPTP in male mice. We examined the expression of Grx1 mRNA and measured the activity of Grx1 in the striatum and midbrain of male and female mice after a single dose of MPTP. mRNA levels and Grx1 activity were significantly increased after a single dose of MPTP treatment in male mice and unaltered in female mice at all time points examined.

3. Redox-sensitive transcription factor AP1 is not activated in female mouse brain regions after MPTP administration

AP1 is a redox-sensitive transcription factor whose activity is modulated by agents that promote intracellular thiol perturbation. AP1 activation was significantly increased in striatum and midbrain of male mice within 30 min after a single exposure to MPTP whereas AP1 activation was unaffected in females vs. untreated con-

¹ These authors contributed equally to the work.

² Correspondence: National Brain Research Centre, Nainwal Mode, Manesar, 122050, Haryana, India. E-mail: vijir@nbrc.ac.in

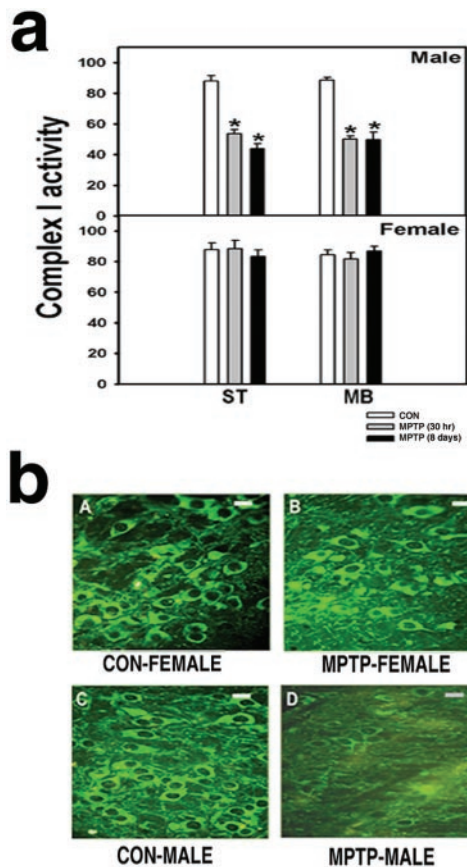


Figure 1. Effect of MPTP on complex I activity (*a*) and on dopaminergic neurons (*b*) in male and female mice. *a*) Male and female mice were administered a single dose of MPTP (30 mg/kg body wt, s.c). Animals were killed after 30 h (gray bars). Animals were also treated with MPTP (30 mg/kg body wt/day, s.c) daily for 8 days (filled bars) and killed 24 h after the last dose. Control animals received vehicle alone (blank bars). Complex I activity was estimated in striatum (ST) and midbrain (MB) of male and female mice and is expressed as nmol of NADH oxidized·min⁻¹·mg protein⁻¹. Values are mean ± SE (*n*=5 animals). *Significantly different from vehicle-treated controls (*P*<0.002). *b*) Immunohistochemical localization of tyrosine hydroxylase in dopaminergic neurons of substantia nigra of male (C, D) and female (A, B) mice after treatment with vehicle (A, C) or MPTP (30 mg/kg body wt/day, s.c) daily for 8 days (B, D). Scale bar = 50 μm.

controls. Thus, the earliest response to MPTP toxicity—activation of AP1—was not seen in female mice whereas in male mice a series of events, starting with AP1 activation 30 min after MPTP administration.

4. Blockade of estrogen receptors by ICI 182,780 renders female mice vulnerable to MPTP toxicity

Sagittal slices of female mouse brain were preincubated with ICI 182,780, an estrogen receptor antagonist (1 nM), for 0.5 h, followed by incubation with MPTP (1 nM) for 1 h. Pretreatment with ICI 182,780 abolished the neuroprotection in females and significant inhibition of complex I activity was seen. When female mice were pretreated with ICI 182,780 (1 mg/kg body wt/

day, s.c) for 15 days and MPTP was administered as a single dose (30 mg/kg body wt, s.c) or daily for 8 days, significant inhibition of complex I activity was seen in striatum and midbrain. Thus, blockade of estrogen receptors by ICI 182,780 abolished the gender difference typically seen after MPTP administration and rendered female mice sensitive to MPTP toxicity.

5. Glutaredoxin expression in female mouse brain is down-regulated by estrogen receptor antagonist ICI 182,780

Constitutive activity of Grx1 in striatum and midbrain of female mice was significantly higher than in males. After blockade of estrogen receptors with ICI 182,780, Grx1 activity in striatum and midbrain of female mice decreased significantly (**Fig. 2b**). ICI 182,780 treatment had no effect on Grx1 expression in liver (**Fig. 2b, LV**).

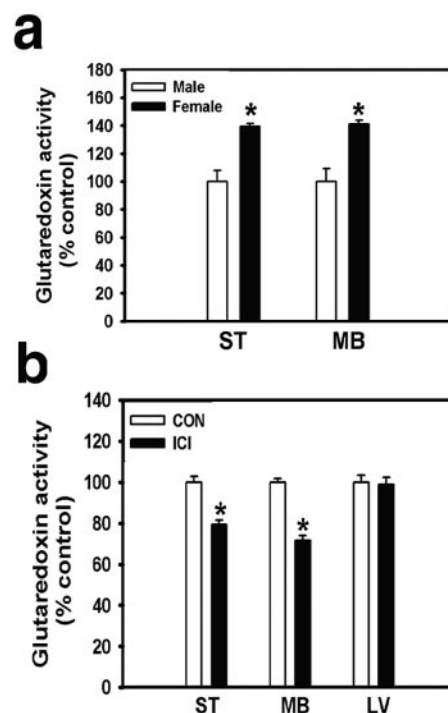
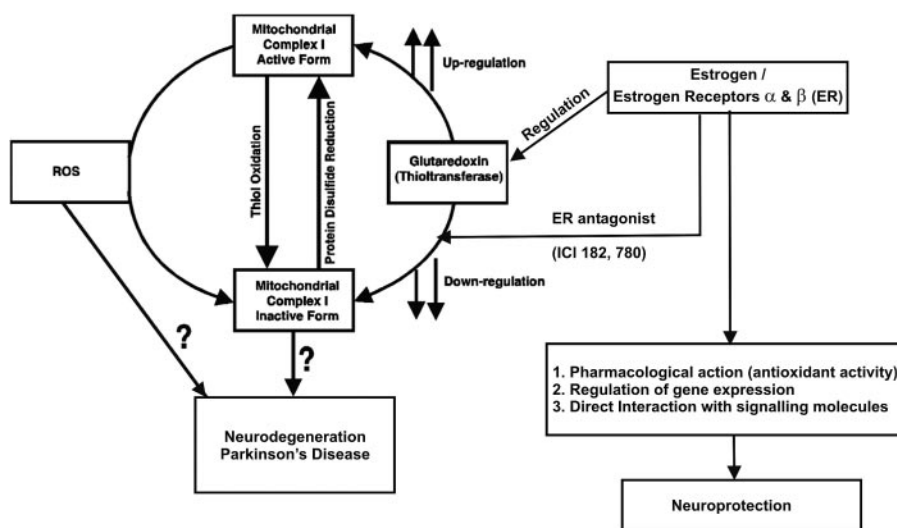


Figure 2. Higher constitutive activity of glutaredoxin in females is down-regulated by estrogen receptor antagonist ICI 182,780 treatment. *a*) Glutaredoxin activity was measured in striatum (ST) and midbrain (MB) of male (blank bars)/female mice (filled bars) and is expressed as % control of corresponding activity in male mouse brain regions. Values are mean ± SE (*n*=3 animals). Glutaredoxin activity in striatum and midbrain from male animals was 17.92 ± 1.40 and 36.45 ± 3.42 nmol of NADPH oxidized·min⁻¹·mg protein⁻¹, respectively. *Values significantly different from corresponding controls (*P*<0.002). *b*) Female mice were treated with ICI 182,780 (1 mg/kg body wt, s.c) daily for 15 days (filled bars); control animals received vehicle alone (blank bars). Glutaredoxin activity was measured in striatum (ST), midbrain (MB), and liver (LV) and is expressed as % control. Values are mean ± SE (*n*=3 animals). *Significantly different from vehicle-treated controls (*P*<0.003).

Figure 3. Schematic illustration depicting the regulation of glutaredoxin (a thiol disulfide oxido-reductase) expression by estrogen and its involvement in neuroprotection related to protein thiol oxidation, leading to mitochondrial complex I dysfunction. Glutaredoxin is essential for maintenance of mitochondrial complex I activity under normal conditions and during oxidative stress. Glutaredoxin activity is higher in females than in males; ICI 182,780, an estrogen antagonist, down-regulates glutaredoxin expression and abolishes the gender difference. Increased activity of glutaredoxin may contribute to the neuroprotection seen in females after MPTP exposure and potentially the dysfunction of mitochondrial complex I commonly seen in Parkinson's disease.



CONCLUSIONS AND SIGNIFICANCE

Female mice are resistant to the mitochondrial dysfunction and dopaminergic cell loss seen in brain regions of male mice after MPTP exposure. In the present study, we have examined the early events after MPTP administration in male and female mice with the aim to identify the critical entities that confer neuroprotection to females. The sequential events observed in striatum and midbrain of male mice after a single dose of MPTP include activation of AP1 after 30 min, followed by loss of GSH, inhibition of mitochondrial complex I and up-regulation of Grx1 mRNA and protein. None of these events were observed in the female mice under similar conditions.

Pretreatment with ICI 182,780, a classical antagonist of α and β estrogen receptors, abolishes the neuroprotection seen in female mice, both in vitro in brain slices and in vivo, and renders them vulnerable to MPTP toxicity. The male mice are unaffected by ICI 182,780, indicating that the observed effect of ICI 182,780 on female mice is through its preferential action on estrogen receptors.

The primary mechanism of MPTP toxicity is through inhibition of complex I leading to mitochondrial dysfunction, followed by cell death. Inhibition of complex I by MPP⁺ involves oxidation of essential thiol groups in the subunits of complex I by glutathionylation since it has several critical thiol groups in its active site. After the neurotoxic insult, Grx1 (which specifically reduces glutathionylated proteins to thiols) is up-regulated and aids recovery of mitochondrial complex I function, and down-regulation of Grx1 prevents the recovery of mitochondrial complex I function after MPTP exposure. Grx1 is critical for maintenance of the mitochondrial complex I under normal conditions and for recovery after neurotoxic insult.

Gender difference is not seen in the constitutive expression of γ -glutamyl cysteine synthetase, the critical enzyme involved in GSH synthesis or in the constitutive levels of GSH. However, Grx1 is expressed constitu-

tively at higher levels in female mice CNS regions, such as striatum and midbrain, than in corresponding levels in males (Fig. 2a). Grx1 activity is down-regulated by pretreating mice with the estrogen antagonist ICI 182,780, indicating that Grx1 expression is regulated through estrogen receptors. The effect of ICI 182,780 on Grx1 was limited to brain regions and not seen in liver although estrogen receptors are present in liver and the expression of several genes is regulated through these receptors. Our observations are in concordance with the fact that protective effect of estrogen is not universal, but restricted to certain tissues such as brain.

Estrogen response element (ERE) sequence is not present upstream of Grx1 gene. However, recent reports have demonstrated that gene expression driven by estrogen receptor could occur through a direct protein-protein interaction of ligand-bound estrogen receptor with transcription factors such as SP1 and EPRE in the absence of ERE. An SP1 binding site is present upstream of the Grx1 gene, indicating such mechanisms could drive Grx1 expression; potentially, this could explain the higher levels of Grx1 in female mouse brain regions.

In the present study we demonstrate the increased expression of Grx1 in female mice brain regions such as striatum and midbrain, which may be involved in estrogen-mediated neuroprotection. It is debatable whether other brain regions such as hippocampus, which are vulnerable to neurotoxic damage (including excitotoxicity) exhibit a similar response. Individual brain regions respond very differently and potentially differential levels of Grx1 expression or turnover may contribute and define the final outcome. In addition to neurotrophic factors, structural proteins and anti-apoptotic factors, thiol disulfide oxido-reductase (Grx1) may represent a new class genes that are regulated by estrogen and are involved in neuroprotection, particularly those related to protein thiol oxidation leading to mitochondrial dysfunction. FJ